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TITLE: Sphingosine-1-Phosphate Receptor Subtype 3: A Novel Therapeutic Target of K-Ras Mutant Driven Non-Small Cell Lung Carcinoma

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14. ABSTRACT: This award aims to characterize the functional role of sphingosine-1-phosphate receptor subtype 3 (S1PR3) in oncogenic *K-Ras* mutant-mediated lung adenocarcinoma (AdC) progression, and to examine the novel therapeutic utility for *K-Ras* mutant-driven lung AdC by targeting S1PR3. There are two specific aims. **Aim 1:** We will use the *LSL-K-RasG12D* mouse model to investigate the role of S1PR3 in the development/maintenance of lung AdC. *LSL-K-RasG12D* will be bred with mice null for S1PR3. $S1PR3^{-/-}$:LSL-K-RasG12D mice will be nasally instilled with adenoviral particle carrying Cre recombinase (Ad-Cre) to induce lung AdC. 3 months later, lung will be weighted, and lung tumor nodules will be quantitated. $S1PR3^{+/+}$:LSL-K-RasG12D mice will be used as a control. **Aim 2:** *LSL-K-RasG12D* mice will be instilled with Ad-Cre. Subsequently, TY-52156, a specific S1PR3 antagonist, will be *i.p.* injected every three days. Mice will be euthanized at 2 and 4 months. Hyperplasia of lung epithelial cells, development of lung adenomas and adenocarcinomas will be assessed. We have completed the animal treatments proposed in Aims 1 and 2. The collected mouse lung specimens are currently being analyzed.

15. SUBJECT TERMS: Oncogenic K-Ras mutant, lung adenocarcinoma, sphingosine-1-phosphate receptor subtype 3

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1. Introduction

Lung cancer forms in tissues of the lung, usually in the cells lining air passages. The two main types of lung cancer are small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). NSCLC comprises about 85% of all lung cancers, and oncogenic *K-Ras* mutant is a feature of more than 25% of NSCLC and represents one of the most prevalent oncogenic drivers in NSCLC. *K-Ras* mutant lung cancers are generally refractory to chemotherapy as well as targeted agent such as EGFR inhibitors. To date, the identification of drugs to therapeutically inhibit *K-Ras* have been unsuccessful, suggesting that other approaches are required. The main goal of this proposal is to characterize the functional role of sphingosine-1-phosphate receptor subtype 3 (S1PR3) in oncogenic *K-Ras* mutant-triggered lung adenocarcinoma (AdC) progression, and to examine the novel therapeutic utility for the treatment of *K-Ras* mutant-triggered lung AdC by targeting S1PR3 receptors. Therefore, completion of this application is expected to provide novel mechanistic insights for the progression of *K-Ras* mutant-triggered NSCLC, particularly in the context of the tumorigenic role of S1PR3 signaling downstream to *K-Ras* activation. This knowledge can be immediately translated into clinical applications.

2. Keywords

Oncogenic K-Ras mutant, lung adenocarcinoma, sphingosine-1-phosphate receptor subtype 3, non-small cell lung cancer

3. Accomplishments

3.1. What were the major goals of the project?

There were two objectives in the proposal.

Objective 1. To determine role of $S1P_3$ receptors in *K-Ras* mutant-triggered lung adenocarcinoma progression in animal (Months 1-6).

1a. Regulatory review and approval of animal protocol (Projective: Months 1-2; Actual: 100% completion).

1b. Mice acquisition and breeding of $S1P3^{-/-}$:LSL-K-Ras^{G12D} and $S1P3^{+/+}$:LSL-K-Ras^{G12D} bi-transgenic mice (Projective: Months 3-4; Actual: 100% completion).

1c. Nasal instillation of adenoviral particles carrying Cre recombinase and lung cancer initiation and development (Projective: Months 5-6; Actual: 20% completion).

1d. Analyze lung cancer specimens of $S1P3^{-/-}$:LSL K-Ras^{G12D} and $S1P3^{+/+}$:LSL K-Ras^{G12D} bi-transgenic mice (Projective: Months 7-8; Actual: 0% completion).

Objective 2. To explore the therapeutic utility of $S1P_3$ antagonist for the treatment of *K-Ras* mutant-triggered lung adenocarcinoma (Months 5-12).

2a. Breeding of LSL K-Ras^{G12D} transgenic mice (Projective: months 5-8; Actual: 100% completion)

2b. Nasal instillation of adenoviral particles carrying Cre recombinase, *i.p.* injection with or without TY-52156, lung cancer initiation and development (Projective: Months 9-10; Actual: 100% completion).

2c. Analyze lung cancer specimens of LSL K-Ras^{G12D} treated with or without TY-52156 (Projective: Months 11-12; Actual: 0% completion).

3.2. What was accomplished under these goals?

Two major activities were achieved in this report period. First, we have successfully generated the S1PR3$^{-/-}$: LSL-K-Ras G12D bi-transgenic mice. The genotyping verification of the generated S1PR3$^{-/-}$: LSL-K-Ras G12D bi-transgenic mice is shown in Figure 1. Secondly, we have treated LSL-KRasG12D mice with or without TY-52156, a specific inhibitor of S1PR3 as proposed in objective 2. Mouse lung tissues were collected, and are currently being analyzed for lung cancer development/ progression. It was taken a longer time than we expect to acquire the LSL-KRas and S1PR3-/- transgenic mice, which delays our time-line of the proposed study.

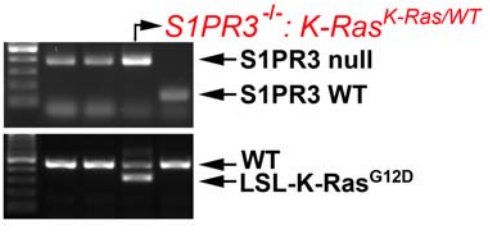

Figure 1. Generation of *S1PR3$^{-/-}$: LSL-K-Ras$^{K-Ras/W}$* bi-transgenic mice

3.3. What opportunities for training and professional development has the project provided?

This project provides training opportunities for Dr. Jiawei Zhao (postdoctoral fellow) and Mrs. Allison Gartung (PhD candidate) to advance their research skills, expertise, and knowledge in the field of lung cancer biology. Mrs. Gartung is expected to defend her dissertation on March 2016.

3.4. How were the results disseminated to communities of interest?

Nothing to Report.

3.5. What do you plan to do during the next reporting period to accomplish the goals?

Two activities are planned. First, the S1PR3$^{-/-}$: LSL-KRasG12D and S1PR3$^{+/+}$: LSL-KRasG12D mice will be nasally injected with Ad-Cre. 2 and 4 months later, mice will be euthanized, and the progression of lung adenocarcinomas will be evaluated. Secondly, we will complete our analysis of lung cancer development in lung tissues collected from LSL-KRasG12D mice injected with or without TY-52156.

4. Impact

4.1. What was the impact on the development of the principal discipline(s) of the project?

Nothing to Report.

4.2. What was the impact on other disciplines?

Nothing to Report.

4.3. What was the impact on technology transfer?

Nothing to Report.

4.4. What was the impact on society beyond science and technology?

Nothing to Report.

5. Changes/Problems

It was taken a longer time than we expect to acquire the LSL-KRas and S1PR3$^{-/-}$ transgenic mice, which delays our time-line of the proposed study. We have requested 6-months no cost extension of this project, and expect to complete this project at the end of no cost extension.

6. Products

6.1. Publications, conference papers, and presentations
Nothing to Report.

6.2. Website(s) or other Internet site(s)
Nothing to Report.

6.3. Technologies or techniques
Nothing to Report.

6.4. Inventions, patent applications, and/or licenses
Nothing to Report.

6.5. Other Products
We have successfully generated the S1PR3$^{-/-}$:LSL-KRasG12D bi-transgenic mice. The novel animal model will provide a unique opportunity to investigate the role of S1PR3 in oncogenic K-Ras mutant-driven lung adenocarcinoma development.

7. Participants & Other Collaborating Organizations
7.1. What individuals have worked on the project?

Name:	Menq-Jer Lee
Project Role:	PD
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1.2
Contribution to Project:	Generation of transgenic mice and design research plan
Funding Support:	

Name:	Jiawei Zhao
Project Role:	MD
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	12
Contribution to Project:	Generation of transgenic mice and treatment with TY-52156
Funding Support:	

Name:	Allison Gartung
Project Role:	PhD candidate
Researcher Identifier (e.g. ORCID ID):	

Nearest person month worked:	3
Contribution to Project:	Generation of transgenic mice and treatment with TY-52156
Funding Support:	

7.2. Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
Nothing to Report.

7.3. What other organizations were involved as partners?
Nothing to Report.

8. Special Reporting Requirements
Nothing to Report.

9. Appendices
Nothing to Report.